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Nutritional status and effect of seaweed chocolate on anemic adolescent girls

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Abstract

The study was carried out to study the nutritional status, develop a product incorporating seaweed and assess its impact on anemic adolescent girls. Five hundred adolescent girls in the age group of 15–18 years were selected from a women's college at Kilakarai, Tamil Nadu, India as the target group to find the prevalence of anemia. Among the 500 subjects 100 with a hemoglobin level of 7–9 g/dL (moderate anemia) were selected by purposive sampling technique for supplementation. Seaweed incorporated chocolate was formulated, standardized, tested for consumer acceptability and in vitro iron bioavailability and supplemented to the selected subjects. The result of the study indicates that seaweed chocolate developed obtained the highest score for overall acceptability, 56 mg of iron/100 g and 11.80 mg of bioavailable iron. The results were found to be promising as there was a significant increase in hemoglobin, TIBC, MCH, MCV, serum iron and serum ferritin levels in the selected subjects. Seaweeds are a less consumed natural resource but abundantly available in the coastal areas of India as they are rich source of nutrients and can be used as an effective therapeutic and nutrient adjunct.

Keywords: Adolescent; Iron deficiency; Anemia; Green seaweed; Bioavailability

1. Introduction

Globalization has led to healthy living consciousness in the most recent years. Simple diet changes have been proven to lower the incidence of non-communicable diseases worldwide. Eating trends of natural food sources have been augmented to bring health and vitality and one such natural resource is seaweeds. Seaweeds are a fascinating and diverse group of organisms which contains treasures for the benefits of human race. Exploiting natural food resources is an easy and quick solution to prevent the rising prevalence of lifestyle and nutritional disorders. Iron deficiency is one of the most prevalent nutritional deficiencies in the world and it affects 20–50% of world population [1]. According to statistics from World Health Organization, 60–70% of Indian adolescent girls are suffering from anemia [2].

Possible ways to improve iron status may include food fortification, diet, anti-helminths treatment and supplementation [3]. Seaweeds are promising natural resources in terms of availability and nutrient density. The benefits of seaweeds are numerous and profound. Harvested in pure seawater, seaweeds can be considered as nature's most complete and balanced nutrient food source [4]. Seaweeds draw an extraordinary wealth of mineral elements from the sea that can account for up to 36% of its dry mass. The mineral macronutrients include sodium, calcium, magnesium, potassium, chlorine, sulfur and phosphorus; the micronutrients include iodine, iron, zinc, copper, selenium, molybdenum, fluoride, manganese, boron, nickel and cobalt. Gram for gram, they are higher in vitamins and minerals than any other class of food. Seaweeds are best used in treating mineral deficiency diseases [5]. *Ulva reticulata* a green seaweed was found to have high iron content and incorporated the seaweed in chocolates, this was done to improve the palatability of the product and chocolate is an all time favorite for all age groups and the ease of packaging and supplementation, chocolate was used as a carrier to supplement the seaweed for anemic adolescent subjects. Moreover it is reported that 40 g chocolate contains six percent of iron recommended for an adult. Hence seaweed chocolate along with grains and nuts were developed

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and assessed its impact on supplementing to anemic adolescent girls.

2. Materials and methods

2.1. Development of seaweed chocolate

The iron content of *U. reticulata* is reported to be 40–50% of the total mineral content. A value added chocolate was developed with the incorporation of seaweed *U. reticulata.* Ethical clearance from the Government Rajaji Medical College and Hospital, Madurai, Tamil Nadu, India was obtained. The ingredients used for the development of seaweed chocolate are bajra, roasted bengal gram, rice flakes, green gram dhal, dry powder of *U. reticulata*, dark chocolate and nuts the selected ingredients are given in Plate 1. The seaweed chocolate was prepared by melting the dark chocolate in a microwave oven for 0.5 s to liquid consistency. Roast and powder all the grains add seaweed powder and half of the nuts to the liquid chocolate, transfer into molds and mix the remaining nuts and freeze for 2 h. Demold the seaweed chocolate and wrap with chocolate paper.

2.2. Standardization of the recipe

Standardization of recipes is a formula specifying the quality of each ingredient required to produce a specific quantity and quality of a particular food item [6].

A written set of description was followed for each recipe. Each ingredient was weighed using a weighing scale before and after preparation. Portion size and duration of preparation were noted in each case. The recipes seaweed chocolate and chocolate were standardized for one serving and repeated thrice to get consistent results. The recipes were then subjected to acceptability tests.

The acceptability test was rated by one hundred female students of a private college, Kilakarai, Ramanathapuram District. The selected subjects were asked to score the product using a score card. A score card is defined as an evaluation card, sample coded with letters or numbers with descriptive terms such as excellent, very good, good, fair and poor. The attributes scored were appearance, color, texture/consistency, flavor and taste [7]. A maximum score of five was given for each attribute.

Plate 1. Ingredients used for preparation of seaweed chocolate with *Ulva reticulata*.

2.3. Estimation of nutrient content and in vitro iron bioavailability of the value added seaweed chocolate

The developed seaweed chocolate was analyzed for nutrients namely carbohydrate [8], protein [9], lipid [10] and iron [11]. The energy values were computed using the nutritive value of Indian foods [12]. The in vitro iron bioavailability was carried out on *U. reticulata* extract, plain chocolate without seaweed and *U. reticulata* incorporated chocolate. This was estimated by dividing dialyzable iron with total iron of the product and expressed as bioavailable iron [13]. *In vitro* iron bioavailability was carried out to understand the percentage of available iron from seaweed since the selected seaweed had a considerably high iron content than any commonly used land vegetables.

2.4. Selection of subjects

A random population of 500 adolescent girls in the age group of 15–18 years studying in a private women's college at Kilakarai, Ramanathapuram district, Tamil Nadu, India was selected to screen for anemia. From those students who were moderately anemic, 100 adolescent girls were selected by purposive sampling. Cyanmethohemoglobin method was used to estimate the blood hemoglobin. The classification given by World Health Organization [14] was applied to categorize the students as mild, moderate and severe anemic category. One hundred adolescent girls, who were moderately anemic were selected by purposive sampling. The one hundred adolescent girls were divided into 50 each with one group serving as the control and the other as the experimental.

2.5. Socio-economic status of the anemic adolescent subjects

An interview schedule was formulated to collect information regarding the socio economic status, age, education and occupational status and income status of the family.

2.6. Assessment of nutritional status

Anthropometric measurements namely height and weight were recorded for all the one hundred anemic adolescents girls. Body mass index (BMI) was calculated by using the formula:

$$\mathrm{BMI} = \mathrm{Weight}\,(\mathrm{kg}) * \mathrm{Height}\,(\mathrm{m}^2)$$

The values were compared with standard percentile chart given by Center for Disease Control [15].

2.7. Dietary habits

Data regarding the nature and quantities of foods consumed were collected using a 24 h recall survey. The 24 h recall method of data collection requires individuals to remember the specific foods and units of foods they consumed in the past 24 h [16]. The raw equivalents was calculated and the nutrients namely energy, carbohydrate, protein, fat, iron, beta carotene and vitamin C was computed by using the Food Composition Table from Nutritive Value of Indian foods given by Indian Council for Medical Research [12]. All the selected 100 adolescent girls were residing in the hostel and followed the same dietary pattern and hence the subjects were on controlled diet.

2.8. Clinical assessment

A clinical assessment schedule was used to elicit details on clinical signs like dry hair, pale pallor, and dryness of mouth and nail pallor. With the help of a medical officer the clinical signs were determined for the 100 adolescent girls.

2.9. Study the impact of the seaweed chocolate among anemic adolescent subjects

The experimental group received 20 g of the seaweed chocolate consisting of *U. reticulata* daily for a period of 120 days. The control group received 20 g chocolate without the seaweed. Before the chocolates were given all the subjects were dewormed. Biochemical analysis was carried out for hemoglobin on the initial, 30th and 120th day. The hemoglobin analysis on the 30th day was carried out in order to find, whether there were any adverse changes in the hemoglobin levels due to supplementation with seaweed chocolate. After the 30th day the supplementation was continued for further 90 days. In addition to hemoglobin, mean corpuscular volume and mean corpuscular, red blood cells, white blood cells, total iron binding capacity, serum iron and serum ferritin were assessed using standard procedures [11].

3. Results

Three hundred and fifty girls had a hemoglobin level below 8 g/dL and 78 students had Hb below 7 g/dL. *U. reticulata* seaweed chocolate had the highest consumer acceptability than the standard chocolate. One hundred grams of seaweed chocolate provided 30 g of carbohydrate, 8.9 g of fat, 10.9 g of protein and 243 kcal of energy. The iron content was high (56 mg) (Table 1). The in vitro iron bioavailability of *U. reticulata* extract and *U. reticulata* chocolate had 11.57 mg of available iron which was less than the available iron of the extract (11.80 mg) and plain chocolate had only 4.56 mg of bioavailable iron. The percentage of bioavailable iron shows that seaweed chocolate had the maximum iron bioavailability of 21%, which is 10% higher than the plain chocolate. Hence chocolate was chosen as an ideal source for incorporating seaweed which will be relished by the young population.

Table 1
Socio economic status of the selected adolescent girls.

Particulars	Control (n = 50)	Experimental (n = 50)
Age (years)		
15–16	24	24
16–17	18	18
17–18	8	8
Type of family		
Nuclear	32	28
Joint	18	22
Family income (Rs.)/month		
1000–5000	10	6
5000–10,000	25	40
10,000–15,000	15	4
Occupation of parent		
Business	10	10
Agriculture	20	19
Government	7	11
Laborers	13	10

Table 2
Body mass index of the selected adolescent girls.

Age wise distribution	BMI[a]	Percentile	Control group (n = 50)	Experimental group (n = 50)
16+	15	<5th	7	8
16+	18	5th–10th	17	16
17+	18	5th–10th	11	13
17+	19	25th	4	4
17+	21	50th	3	1
18+	18	5th–10th	5	6
18	25	85th	3	2

[a] CDC standards (2000).

The socioeconomic status of the selected adolescent girls is given in Table 2. Thirty-nine percent of the subject's parents were agriculturists. The family income for 65% of adolescents ranged between Rs. 5,000 and Rs. 10,000. Table 3 indicates the age of puberty of the selected adolescent anemic girls. The body mass index of the selected adolescent girls is presented in Table 4. It was found that 15 girls were underweight and 80% of the girls were in the fifth to 50th percentile. Only five girls were in the 85th percentile indicating that they have a risk of being overweight. The dietary pattern of the selected girls showed that 66% of the subjects were non-vegetarians, 19% were ova-vegetarian and 15% were vegetarians (Table 5). It is clear from the results in Table 6 that the selected girls had a deficit intake of the proximate nutrients namely energy and protein, micro-nutrients calcium, iron, vitamins, beta carotene and vitamin C when compared to that of the ICMR Recommended Daily Allowance. Fat intake was higher by 5.2–5.7% than the

Table 3
Age at puberty.

Age (years)	Control group (n = 50)	Experimental group (n = 50)
8–11	20	20
11–12	12	6
12–13	8	14
13–14	10	10

Table 4
Nutrient intake of the selected adolescent girls.

Nutrients	RDA ICMR	Mean nutrient intake			
		Control ($n = 50$)	Percent difference	Experimental ($n = 50$)	Percent difference
Energy (kcal)	2060	1842.76	−10.54	1836	−10.81
Protein (g)	63	47.25	−25	50	−20.6
Fat (g)	22	23.15	+5.2	23.25	+5.7
Calcium (mg)	500	392.56	−21.49	400.28	−19.9
Iron (mg)	30	20.62	−31.27	21.23	−31.26
Carotene (μg)	2400	1304.07	−45.66	1376.08	−45.6
Vitamin C (mg)	40	20.76	−48.09	20.89	−48.1

Table 5
Clinical examination of the selected adolescent girls.

Clinical signs[a]	Control group ($n = 50$)	Experimental group ($n = 50$)
Dry hair	12	23
Pale eye pallor	26	28
Dryness of skin	12	10
Dryness of mouth	29	22
Pale pallor	32	21
Pale nail Pallor	32	29

[a] Multiple signs.

Table 6
Nutrient content of the seaweed chocolate.

Nutrients	*Ulva reticulata* incorporated chocolate
Carbohydrate (g)	30
Fat (g)	8.9
Protein (g)	10.9
Energy (kcal)[a]	243
Iron (mg)	56

[a] Computed values.

Recommended Daily Allowance in both groups. All the samples selected were residents of the college hostel and consumed a cycle menu which may be the reason for the deficit of above said nutrients in their diets. The clinical examination of the selected adolescent girls is presented in Table 6 and reveals that majority (32 girls in control) and (29 girls in the experimental group) had pale pallor and pale nail pallor respectively which relate to iron deficiency. Twenty-six and twenty-eight girls were observed to have pale eye pallor. All the above-mentioned clinical signs are indicative of anemia.

The impact of seaweed chocolate used as a nutritional supplement on the blood parameters is presented below in Table 7

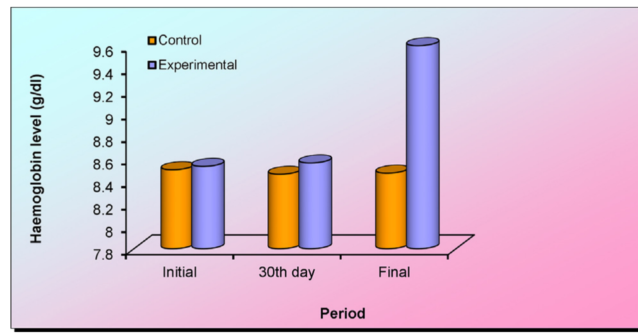

Fig. 1. Impact of seaweed chocolate on hemoglobin level (g/dl) of the selected adolescent girls.

and Fig. 1. Table 7 indicates the effect of seaweed chocolate on hemoglobin levels of the selected girls after a period of 120 days. The result of the study reveals that the mean hemoglobin level has significantly increased in the experimental group than the control group after 120 days of supplementation. Though the increase in hemoglobin was very negligible during the period of one month it increased from 8.53 ± 0.27 to 9.60 ± 0.59 in experimental group. On an average there is 1.07 g increase in the blood hemoglobin level after 120 days. The paired t value of 25.645 is higher than the table value of 2.807 at 1% level of significance. The details on mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) of the selected adolescent girls in control and experimental groups are given in Table 8. The initial values of MCV show that all the selected adolescent girls had the average blood cell volume below the normal range. Among the two groups all the subjects in the experimental group showed an increase in MCV of 74.47 ± 2.61 to 84.39 ± 3.37. The "t" value shows a significant result. The impact of seaweed chocolate on mean corpuscular hemoglobin values (MCH) point

Table 7
Impact of seaweed chocolate on hemoglobin level (g/dl) of the selected adolescent girls.

Period	Control ($n = 50$)	Experimental ($n = 50$)
Initial	8.50 ± 0.33	8.53 ± 0.27
30th day	8.46 ± 0.31	8.56 ± 0.06
Final	8.47 ± 0.34	9.60 ± 0.59
't'-Test	1.06^{NS}	25.645**

Groups compared between initial and final period; $^{NS}(t > 0.05)$ – not significant, **($t < 0.01$) significant at 1% level.

Table 8
Impact of seaweed chocolate on mean corpuscular volume and mean corpuscular hemoglobin of the selected adolescent girls.

Parameters	MCV (cu microns)		MCH (mcg)	
	C ($n = 50$)	E ($n = 50$)	C ($n = 50$)	E ($n = 50$)
Initial	71.16 ± 0.33	74.47 ± 2.61	20.44 ± 3.37	22.49 ± 2.79
Final	69.66 ± 2.41	84.39 ± 3.37	20.70 ± 3.72	27.59 ± 2.76
't' value	3.362**	17.76**	1.34^{NS}	11.78**

C – control group; E – experimental group; $^{NS}(t > 0.05)$ – not significant, **($t < 0.01$) significant at 1% level.

Table 9
Impact of seaweed chocolate on RBC and WBC of the selected adolescent girls.

Period of supplementation	RBC ($\times 10^6$ µL)		WBC ($\times 10^3$ µL)	
	C ($n=50$)	E ($n=50$)	C ($n=50$)	E ($n=50$)
Initial	4.4	4.3	6.5	6.0
Final	5.1	5.4	6.8	6.9
Difference	0.7	1.1	0.3	0.9

C – control group; E – experimental group.

out that initially the subjects in both control and experimental group had values below normal ranging from 20.44 ± 3.37 to 22.49 ± 2.79 µg. The paired "t" value is 17.76 for MCV and 11.78 for MCH for the experimental group, which is higher than the table value of 2.807 at 1% level of significance. Table 9 shows the mean red blood cell and white blood cell count of the selected adolescent girls in control and experimental groups before and after supplementation.

The increase in red blood cell count ranged from 0.7 to 1.1 in the control and experimental group respectively. In the experimental group the initial value was 4.3 and it increased to 5.4. The WBC count of the subjects in experimental group increased from 6.0 to 6.9 × 103 µL. Table 10 shows the total iron binding capacity (TIBC) and serum iron of the experimental and control groups selected for the study. Results of the study revealed that there is a significant increase in TIBC levels of the experimental group than the control group. The TIBC levels increased from 397.84 ± 18.1 to 423.38 ± 12.36 in the experimental groups, whereas in the control group there was a negligible increase from initial to final as shown in the paired mean difference as 0.644 ± 4.6. A significant difference between the initial and final values of mean serum iron levels is noted. This shows there is a significant difference ($t < 0.01$) between initial and final values of serum iron. Table 11 shows the serum ferritin levels of the experimental and control groups selected for the study. From the table it can be inferred that there was 2.63 ± 2.5 paired mean difference in serum ferritin levels of the experimental group whereas there was not much change in the level of serum ferritin in control group. The results are statistically significant at 1% level of significance for the experimental groups. The control and experimental values of all the blood parameters namely hemoglobin, Mean Corpuscular Volume, Mean Corpuscular Hemoglobin, Total Iron Binding Capacity, serum iron and serum ferritin were compared in order to find out whether there is a significant difference in the mean values of the two groups and the details are shown in Table 12. Analysis of co-variance (ANACOVA) was applied to find whether the blood parameters

Table 11
Impact of seaweed chocolate on serum ferritin of the selected adolescent girls.

Period of supplementation	Serum ferritin (µg/L)	
	C ($n=50$)	E ($n=50$)
Initial	17.73 ± 6.07	15.20 ± 3.05
Final	7.76 ± 5.75	17.84 ± 4.71
t-Test	0.461^{NS}	7.40**

C – control; E – experimental; ^{NS}($t > 0.05$) – not significant, **($t < 0.01$) significant at 1% level.

final values of control and experimental groups varied significantly. The results of ANACOVA shows that the covariate of all the blood parameters initially had a significant effect at 1% level on post values of all the selected blood parameters. The corresponding F-value shows there is a significant difference between control and experimental group in the final values.

4. Discussion

Prevalence of anemia among adolescent girls is found to be very high in India. Preventive measures are fewer than curative programs thus simple diet modification by judicial use of locally available iron rich foods forms a pathway for prevention and cure of nutritional deficiencies in developing countries. Thus in the study locally available ingredient found to have bioavailable iron was selected for supplementation to anemic adolescence.

Seaweeds have all essential minerals ounce for ounce it is higher in minerals than any other class of food [17]. Seaweeds are also one of the most important vegetable sources of iron [18]. The percentage of bioavailable iron shows that seaweed chocolate had the maximum iron bioavailability of 21%, which is 10% higher than the plain chocolate. United States Department of Agriculture reveals that 40 g of dark chocolate has 6% iron from that of the Recommended Dietary Allowance (RDA) required for adult [19] hence seaweed chocolate was prepared for supplementation.

The assessment of nutritional status of the selected adolescents revealed that anemia is highly prevalent among the selected subjects and the selected moderately anemic subjects were not only undernourished but few were also obese. The etiology of anemia in obese individuals is uncertain but may be related to low-quality diets or increased needs relative to body weight [20]. The subjects income and anemic condition were also found to be correlated. A study showed that the prevalence rates of anemia and iron deficiency were 39% and 62%, respectively, in young

Table 10
Impact of seaweed chocolate on TIBC and serum iron of the selected adolescent girls.

Period of supplementation	Total iron binding capacity (mg/dL) (TIBC)		Serum iron (µg/dL)	
	C ($n=50$)	E ($n=50$)	C ($n=50$)	E ($n=50$)
Initial	373.11 ± 26.09	397.84 ± 18.1	23.04 ± 1.6	23.20 ± 0.99
Final	373.76 ± 23.5	423.38 ± 12.36	23.24v± 1.8	27.88 ± 1.60
t-Test	0.978^{NS}	15.40**	2.77**	17.5**

C – control group; E – experimental group; ^{NS}($t > 0.05$) – not significant, **($t < 0.01$) significant at 1% level.

Table 12
Comparison of blood parameters between control and experimental group.

Blood parameters	Adjusted mean		F-value	Level of significance
	Control ($n = 50$)	Experimental ($n = 50$)		
Hemoglobin (g/dL)	8.481	9.591	216.422	$P < 0.01$
MCV (cu.m)	70.10	83.95	7.766	$P < 0.01$
MCH (mcg)	21.48	26.80	108.939	$P < 0.01$
TIBC (μg/dL)	383.21	413.87	55.709	$P < 0.01$
Serum iron (μg/dL)	23.30	27.82	62.733	$P < 0.01$
Serum ferritin (μg/L)	16.49	19.13	670.023	$P < 0.01$

$P < 0.01$ – significant at 1% level.

women and adolescents of low socioeconomic status in India [21].

The impact of supplementation of seaweed chocolate on blood parameters was studied and the results are as follows the increase in hemoglobin may be attributed to the high iron content present in *U. reticulata* (56 mg/100 g). The seaweed extract also has a high iron bioavailability. Statistically there is a significant difference between the initial and final mean hemoglobin levels for experimental group. Supplementing diets with seaweeds may help to improve the iron status. It is reported iron from algae are easily absorbed by the body [22]. On the other hand after use of seaweed chocolate there was a significant increase in the MCH values in the experimental group (27.59 ± 2.76 mg). This result may be attributed to the high amount of iron and other micronutrients present in *U. reticulata* which is required for maintenance of blood volume. The mean corpuscular volume indicate the volume occupied by the average red blood cells and the optimum range is 82–89.9 cu.m. Statistically there is a significant difference between initial and final mean of MCV and MCH for experimental groups.

Owing to the increase in white blood cell counts in experimental group it can be concluded that seaweeds can act as immune enhancers. A study on the anti-HIV-active and anti-herpes activity of brown seaweed *Fucus vesiculosus*, red algae *Dumontia* and *U. rigida* green alga was due to the presence of soluble and insoluble dietary fiber, phyto-nutrients and essential trace minerals [23]. The normal level of TIBC is an indicator that the subjects have an improved iron status.

The impact of seaweed chocolate on serum iron proved to be beneficial pointing out that there is an increase in serum iron in the experimental group and a mild increase in control group. This result points out a gradual increase might be possible in the serum iron status if supplementation is given for a longer duration. Diet provided to 61 men with daily inclusion of spinach, seaweed, organ meats and shellfish showed higher levels of serum iron, serum selenium and copper among the selected men [24]. Similar result is obtained in the present study. Thus seaweed can act as a potential supplement to combat iron deficiency. Decrease in serum ferritin can be correlated with need for iron in the body and decrease in serum ferritin levels are indicators of iron deficiency [25]. These results confirm that seaweed added in small amounts can be beneficial in overcoming iron deficiency anemia. Research studies supports that seaweeds are better source of bioavailable iron. One tablespoon of dried seaweeds contains between 1/2 mg and 35 mg of bioavailable iron [26].

5. Conclusion

Seaweeds have been consumed by people dating back to 100 centuries but they have never found their rightful place in the daily diets of our population. The consumption of seaweed is sporadically seen near the coastal areas but is still underexploited. Seaweed products are used commercially but as a natural food the goodness of seaweeds have to be utilized. The research points out that seaweed contains substantial amount of protein, fat, carbohydrate and essential minerals iron, calcium, selenium, phosphorus, zinc, sodium and magnesium and essential vitamins C and A. The therapeutic effect of seaweeds shows a promising result. Seaweeds could be an ideal food adjunct and be used to combat iron deficiency at large. As nature's wealth seaweeds have to be used to promote future health.

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